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Determination of Soya Protein in Processed Foods

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ABSTRACT

Many qualitative and quantitative analytical procedures for determining vegetable proteins in processed foods have been studied by researchers throughout the world, but each technique seems to have limitations. Several analytical procedures that have potential for both qualitative and quantitative determination of soya protein in foods are reviewed.

INTRODUCTION

Processed foods may contain added vegetable protein for a number of reasons. Most commonly the supplements act as binders; they are added for improvement of texture and nutrition or for retention of water and/or fat. Soya protein can be incorporated into foods as defatted flour, a concentrate, of an isolate. Complications arise in the analysis of food products that contain the soya protein, because commercially available products can be obtained in texturized forms that may be artificially colored and fortified with vitamins and minerals. The food technologist procures these products, mixes them with other ingredients, and manufactures a product. During processing, proteins interact both chemically and physically with other components to form intricate composites. This mass is then given to the analyst to determine the amounts of additives introduced into the food.

Since most food products in the United States and other countries must meet standards of identity, it has been necessary to develop methods that will detect and quantitate vegetable protein products in foods. Two excellent reviews on the determination of vegetable proteins have recently been published (1,2).

MICROSCOPY AND HISTOLOGICAL METHODS

Probably the oldest, best known microscopy method is inspection for characteristic hourglass and/or palisade cells in the residue that remains after extracting with potassium hydroxide (3). Determining the presence of calcium oxalate crystals in the soybean cotyledon cells (4) has also been used as a qualitative test to detect soya meal or a textured soya meal in meat products (5).

Pomeranz and Miller (6) developed a method that enables one to detect soya flour in wheat flour by observing the canary-yellow fluorescence of soybean particles viewed under ultraviolet light (360 mμ) with low magnification. The smallest quantity of soya flour determined was 0.01%.

If histological stains are used, more elaborate methods exist that enable the measurement not only of carbohydrates but also of proteins. Specifically, detection and even quantitative approximation can be made of textured soya flour (TSF). Smith (7) suggests four useful stains: toluidine blue, iodine, periodic acid/Schiff reagent, and acridine orange. Coomaraswamy and Flint and Meech (8,9) quantitated TSF added to meat products by using a toluidine blue stain; they measured TSF with a standard deviation of 1.85% at the 45% level of addition. They reported that an experienced person can analyze one or two samples per day, a rate too slow for routine screening. Concentrates or isolates cannot be determined because the amount of carbohydrate present in these products is variable. That is, a defatted soya flour has 29% carbohydrate, a soya concentrate has 16%, and an isolate may contain only 2% carbohydrate (10). Consequently, it is necessary to know what type of product is present, and the techniques are not applicable when more than one type is added to the food item. However, Parisi et al. (11) in Italy claim that they can detect soya flours, concentrates, and isolates in commercial meat products by the periodic acid-Schiff base reaction, which is dependent on the presence of carbohydrate.

Bergeron and Durand (12), using several protein stains, developed a histological technique that is reported to be rapid and capable of detecting as little as 1% soybean protein in meat products. They report satisfactory results with fresh, heated or putrefied meat containing soy flour, concentrates or isolates.

IMMUNOCHEMICAL ANALYSIS

Immunological techniques should be the best procedures for the determination of nonmeat proteins in meat products because of the high specificity of antibodies and the sensitivity of the antigen-antibody reaction. By having several different antibodies available, i.e., for casein, wheat, corn, and so on, a researcher or analyst should be able to determine which substances have been added to food products. An excellent review of the literature in this field has recently been published by Olsman and Hitchcock (2). Since much of the immunological research has been done in Europe, they have done an outstanding job of providing a review of the European journals, which may not be available to everyone.

Poli et al. (13) recently reported a unique crossover electrophoresis technique that uses antisera. In the procedure, the unknown protein sample is solubilized in buffer containing sodium dodecylsulphate and mercaptoethanol, and the migrated against a soy-specific rabbit antisera. The resultant precipitin band may be enhanced with a sheep anti-rabbit gamma globulin that has been coupled to a fluorescent compound. The arcs of precipitation are observed as fluorescent bands.

Another reaction or technique (14) that may be useful in the determination of nonmeat protein in food systems is the "enzyme-linked immunosorbent assay" (ELISA), where enzyme is coupled to the antibody, thus enabling the analyst to develop a quantitative colorimetric assay for the amount of antigen-antibody present in a given system.

In immunological techniques, the major problem is quantitative reaction of antibodies from undenatured proteins with denatured (heated) proteins. Several authors (15-17) have reported that heat treatments are detrimental to the quantitation of foreign proteins.

West German researchers have recently reported (18-20) that antigenicity is lost when soya protein is heated to 120 C for 50 min. However, the heated proteins or isolated polypeptide chains can be conjugated with a carrier protein, and corresponding antibodies prepared. These antibodies may then be applied to detect heated soya protein in various products.

In 1978, Koh (21) published an interesting article describing the identification and quantitation of the amount of soya protein added to both cooked and uncooked beef mixtures. Koh cooked products to 71 C internal temperature and analyzed the mixtures by immunoelectrophoresis; he used an unusual technique of preparing antibodies from a "renatured" protein. This approach needs to be investigated further, and the quantitation should be studied thoroughly.

ELECTROPHORESIS

For electrophoresis to be a successful tool in detection of vegetable proteins in processed foods, the proteins must first be dissolved. The analyst is often faced with extracting the protein from an insoluble matrix. Extraction of the protein can be nearly complete when heat, urea, guanidine or detergent are used in the presence of a reducing agent such as mercaptoethanol. Lee et al. (22) were able to solubilize 95% of the protein in soya-beef blends, which had been heated to 100 C for 1 hr, with tris-HCl buffer containing 3% sodium dodecylsulfate (SDS) and 1% mercaptoethanol.

In 1972, Parsons and Lawrie (23) identified an electrophoretic protein band unique to soya and a protein band characteristic of meat. They quantitated soya added to meat products by measuring the areas of the two bands densitometrically. Essentially the same procedure has been used by others (24-25).

Persson and Appleqvist (26) used polyacrylamide gel electrophoresis with SDS in the buffer rather than the urea used by Parsons and Lawrie. Thus, they were able to measure the amount of soya protein added to hamburger-type foods. Persson and Appleqvist used a different set of protein bands for quantitation than did earlier researchers (22-25).

Another approach has been reported by Fischer and Belitz (27), who used two protein bands to identify soya. They isolated the second protein band and determined its amino acid composition, N-terminal amino acid, isoelectric point and molecular weight.

All procedures that involve the use of electrophoretic bands, especially in the presence of urea or SDS, appear to be very effective systems for determining extraneous protein products in processed foods; they deserve more effort and, probably, collaborative studies. The use of electrophoresis combined with immunological techniques seems to have great possibilities.

NEW APPROACHES

Several new approaches to the determination of soya in processed foods have been described within the last 2-3 years. Bailey and coworkers (28-31) have isolated and characterized a unique penta peptide (Ser-Glu-Glu-Ala-Arg), which they obtained by tryptic hydrolysis of the major soy protein—11S globulin after it had been thoroughly denatured. This method has a definite advantage because the 11S protein is not lost when soybean flour is fractionated into concentrate or isolate. In their latest paper (31), these authors identified unique peptides from both soy and meat. By using both the soya and meat peptide peaks (in the case of a mixture) from the chromatogram, simultaneous estimation of both soya and meat can be made.

Another approach that may have some promise in estimating the amount of nonmeat protein in meat blends is based on computer comparisons of the total amino acid pattern, as described by Lindqvist et al. (32). The Swedish authors prepared a mixture containing 54% milk protein, 36% whey protein and 10% soya protein. After amino acid analysis of the blend and a stepwise multiple-regression analysis with a computer, they concluded that their mixture contained 50% milk, 37% whey, and 13% soya protein. Researchers in the Netherlands (33) have tried this technique to measure qualitatively the amount of various proteins added to extended meat products. The results look interesting, but whether good qualitative data can be obtained will have to be decided later.

One method that we have investigated (34), which may have limited use in developing countries, depends on the fact that certain materials in soya products fluoresce at 440 nm when excited at 360 nm. The method involves a simple extraction, filtration and measurement of the fluorescence of the solution. The procedure does not seem to work on cooked products because additional fluorescent materials form during cooking.

Another study we made involved measuring the carbohydrates in soya (10). One of the proteins of soya, the 7S protein, has been studied and found to contain ~4% mannose that is covalently bound to the protein. The analysis of soya for mannose was successful, but the error in measuring a small amount of the sugar was too large for any quantitative work. Carbohydrate analysis of soya products did not indicate unusual carbohydrates except for pinitol, which apparently is not covalently bound because its concentration varies during processing.

MISCELLANEOUS METHODS

Many studies have been attempted to solve the problem of determining the amount of vegetable protein added to processed foods. Quantitative procedures that have been investigated but do not really solve the problems are $^{13}C:^{12}C$ isotopic ratios (35), metal and fiber analysis (36), determination of phytate (37) and analysis for conavanine (38).

Several physical separations have been tried that have met with little success. These are density gradient separations (39-41), high-performance liquid chromatography (42), isoelectric focusing (42), and most recently, gel permeation chromatography of a carbohydrate in soya, which is not digested by amylase (43).

In addition, TiO_2 has been added in the manufacture of some soybean protein isolates to serve as a "Tag" (44), and attempts have even been made to measure the quantity of meat (45-46), thus obtaining the amount of nonmeat in a product by difference.

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